

<b>11 COCAINE, COCAETHYLENE AND BENZOYLECGONINE QUANTITATION AND CONFIRMATION BY SPE AND GCMS</b>	Page 1 of 5
<b>Division of Forensic Science  TOXICOLOGY TECHNICAL PROCEDURES MANUAL</b>	Amendment Designator:
	Effective Date: 31-March-2004
<p style="text-align: center;"><b>11 COCAINE, COCAETHYLENE AND BENZOYLECGONINE QUANTITATION AND CONFIRMATION BY SPE AND GCMS</b></p> <p><b>11.1 Summary</b></p> <p>11.1.1 Biological samples are extracted using United Chemical Technologies® solid phase extraction columns. The extracts are concentrated, treated with a derivatizing reagent and injected into the GCMS for confirmation and quantitation by selected ion monitoring.</p> <p><b>11.2 Specimen Requirements</b></p> <p>11.2.1 2 mL of whole blood, biological fluids or tissue homogenates.</p> <p><b>11.3 Reagents And Standards</b></p> <p>11.3.1 Cocaine, cocathylene and benzoylecgonine, 1 mg/mL</p> <p>11.3.2 Cocaine-d<sub>3</sub> and/or benzoylecgonine-d<sub>3</sub>, 100 µg/mL</p> <p>11.3.3 Concentrated Acetic Acid</p> <p>11.3.4 Methanol</p> <p>11.3.5 Hexane</p> <p>11.3.6 Dichloromethane</p> <p>11.3.7 Isopropanol</p> <p>11.3.8 Acetonitrile</p> <p>11.3.9 Concentrated Ammonium Hydroxide</p> <p>11.3.10 N-Methyl-N-([tert-butyldimethyl-silyl]trifluoroacetamide (MTBSTFA) or N,O bis (Trimethylsilyl) trifluoroacetamide (BSTFA) with 1% TMCS</p> <p>11.3.11 Ethyl acetate</p> <p><b>11.4 Solutions, Internal Standards, Calibrators, Controls</b></p> <p>11.4.1 1 M Acetic Acid. Add 100-200 mL dH<sub>2</sub>O to a 1L volumetric flask. Add 57.5mL glacial acetic acid. QS to volume with dH<sub>2</sub>O.</p> <p>11.4.2 Dichloromethane/isopropanol, 80:20 (v:v): mix 800 mL dichloromethane and 200 mL isopropanol.</p> <p>11.4.3 When using UCT CleanScreen® SPE Extraction columns, either sodium or potassium phosphate buffer may be used. However, the same buffer (sodium or phosphate) must be used throughout the duration of the procedure.</p> <p>11.4.4 0.1M Potassium Phosphate Buffer, pH 6.0. Weigh out 13.61 g of KH<sub>2</sub>PO<sub>4</sub> and transfer into a 1 L volumetric flask containing approximately 800 mL of dH<sub>2</sub>O. Adjust the pH of the above solution to 6.0 by the addition of 5 M potassium hydroxide while stirring. QS to volume with dH<sub>2</sub>O. Solution can also be purchased (e.g. Fisher).</p>	

<b>11 COCAINE, COCAETHYLENE AND BENZOYLECGONINE QUANTITATION AND CONFIRMATION BY SPE AND GCMS</b>	Page 2 of 5
<b>Division of Forensic Science  TOXICOLOGY TECHNICAL PROCEDURES MANUAL</b>	Amendment Designator:
	Effective Date: 31-March-2004
<p>11.4.5 0.1M Sodium Phosphate Buffer, Ph 6.0. Weigh out 1.70g Na<sub>2</sub>HPO<sub>4</sub> and 12.14g NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O and transfer to a 1 L volumetric flask containing approximately 800 mL dH<sub>2</sub>O. Adjust the pH of the above solution to 6.0 by the addition of 5 M sodium hydroxide. QS to volume with dH<sub>2</sub>O. Solution can also be purchased (e.g. Fisher).</p> <p>11.4.6 Dichloromethane/Isopropanol/Ammonium Hydroxide elution solvent: Mix 78 mL dichloromethane with 20 mL isopropanol. Mix well. In hood, add 2 mL of concentrated NH<sub>4</sub>OH. Mix gently.</p> <p>11.4.7 Working standard solutions</p> <p>11.4.7.1 100 µg/mL cocaine, cocaethylene and benzoylecgonine: Pipet 1.0 mL each of the stock 1 mg/mL solution of cocaine, cocaethylene and benzoylecgonine into a 10 mL volumetric flask and QS to volume with methanol.</p> <p>11.4.7.2 10 µg/mL cocaine, cocaethylene and benzoylecgonine: Pipet 1.0 mL of the 100 µg/mL solution of cocaine, cocaethylene and benzoylecgonine into a 10 mL volumetric flask and QS to volume with methanol.</p> <p>11.4.7.3 Working internal standard solution, 10 µg/mL cocaine-d<sub>3</sub> and/or benzoylecgonine-d<sub>3</sub>: Pipet 1.0 mL of the 100 µg/mL stock solutions of cocaine-d<sub>3</sub> and/or benzoylecgonine-d<sub>3</sub> in a 10 mL volumetric flask and QS to volume with methanol.</p> <p>11.4.8 The following are examples of acceptable procedures for the preparation of calibrators. Other quantitative dilutions may be acceptable to achieve similar results.</p> <p>11.4.8.1 Cal 1: 2.5 mg/L: 500 µL of 10 µg/mL working standard + 2.0 blank blood</p> <p>11.4.8.2 Cal 2: 1.0 mg/L: 200 µL of 10 µg/mL working standard + 2.0 blank blood</p> <p>11.4.8.3 Cal 3: 0.5 mg/L: 100 µL of 10 µg/mL working standard + 2.0 blank blood</p> <p>11.4.8.4 Cal 4: 0.25 mg/L: 50 µL of 10 µg/mL working standard + 2.0 blank blood</p> <p>11.4.8.5 Cal 5: 0.05 mg/L: 10 µL of 10 µg/mL working standard + 2.0 blank blood</p> <p>11.4.8.6 Cal 6: 0.02 mg/L: 4 µL of 10 µg/mL working standard + 2.0 blank blood</p> <p>11.4.8.7 Cal 7: 0.01 mg/L: 2 µL of 10 µg/mL working standard + 2.0 blank blood</p> <p>11.4.9 Prepare the first standard by adding 200 µL of the 100 µg/mL to 4.8 mL blank blood to give a final concentration of 4 mg/L.</p> <p>11.4.9.1 Cal 1: 4.0 mg/L: 2 mL of 4 mg/L standard</p> <p>11.4.9.2 Cal 2: 1.5 mg/L: 750µL of 4 mg/L standard + 1.25mL blank blood</p> <p>11.4.9.3 Cal 3: 0.5 mg/L: 250µL of 4 mg/L standard + 1.75mL blank blood</p> <p>11.4.9.4 Cal 4: 0.25 mg/L: 125µL of 4 mg/L standard + 1.85mL blank blood</p> <p>11.4.9.5 Cal 5: 0.10 mg/L: 50µL of 4 mg/L standard + 1.9mL of blank blood</p> <p>11.4.9.6 Cal 6: 0.05 mg/L: 25µL of 4 mg/L standard + 2 mL of blank blood</p>	

11 COCAINE, COCAETHYLENE AND BENZOYLECGONINE QUANTITATION AND CONFIRMATION BY SPE AND GCMS		Page 3 of 5
Division of Forensic Science  TOXICOLOGY TECHNICAL PROCEDURES MANUAL		Amendment Designator:
		Effective Date: 31-March-2004
11.4.9.7 Cal 7: 0.02 mg/L: 10µL of 4 mg/L standard + 2 mL of blank blood		
11.4.10 Controls		
11.4.10.1 Negative blood control. Blood bank blood (or comparable) determined not to contain cocaine, cocaethylene or benzoylecgonine.		
11.4.10.2 QAS Toxicology Control: 0.1 mg/L cocaine and cocaethylene and 1.0 mg/L benzoylecgonine		
11.4.10.3 In house control is prepared from a different lot number or a different manufacturer of cocaine, cocaethylene and benzoylecgonine.		
11.5 Apparatus		
11.5.1 Agilent GC/MSD, Chemstation software, compatible computer & printer		
11.5.2 Test tubes, 16 x 125 mm round bottom, screw cap tubes, borosilicate glass with Teflon caps		
11.5.3 Test tubes, 16 x 125 mm round bottom tubes, borosilicate glass		
11.5.4 Test tubes, 16 x 114 mm (10 mL) glass tubes, conical bottom		
11.5.5 Centrifuge capable of 2,000 – 3,000 rpm		
11.5.6 Cleanscreen® Extraction Cartridges (ZSDAU020) from United Chemical Technologies (200 mg columns)		
11.5.7 Solid phase extraction manifold		
11.5.8 Vortex mixer		
11.5.9 Heating block		
11.5.10 Evaporator/concentrator		
11.5.11 GC autosampler vials and inserts		
11.5.12 HP GC/MSD		
11.5.12.1 Acquisition Mode: SIM		
11.5.12.2 Cocaine: <u>303</u> , 198, 272 Alternate ions: <u>182</u> , 303, 198		
11.5.12.3 Cocaine-d <sub>3</sub> : <u>306</u> , 185		
11.5.12.4 Cocaethylene: <u>317</u> , 196, 272 Alternate ions: <u>82</u> , 196, 317		
11.5.12.5 Benzoylecgonine: MTBSTFA ions <u>403</u> , 282, 346 BSTFA ions: <u>240</u> , 361, 256		
11.5.12.6 Benzoylecgonine-d <sub>3</sub> : MTBSTFA ions <u>406</u> , 285 BSTFA ions: <u>85</u>		
11.5.12.7 Column: HP 5MS 25 m x 0.25 mm x 0.25 µm		
11.5.12.8 Detector Temperature: 280° C		

<b>11 COCAINE, COCAETHYLENE AND BENZOYLECGONINE QUANTITATION AND CONFIRMATION BY SPE AND GCMS</b>	Page 4 of 5
<b>Division of Forensic Science  TOXICOLOGY TECHNICAL PROCEDURES MANUAL</b>	Amendment Designator:
	Effective Date: 31-March-2004
<p>11.5.12.9 Instrument conditions may be changed to permit improved performance.</p> <p>11.5.12.9.1 Oven Program</p> <ul style="list-style-type: none"> <li>• Equilibration time: 0.50 minutes</li> <li>• Initial temp: 130° C</li> <li>• Initial time: 1 minutes</li> <li>• Ramp: 17° C/min</li> <li>• Final Temp: 280° C</li> <li>• Final Time: 7 minutes</li> <li>• Run Time: 17 minutes</li> </ul> <p>11.5.12.9.2 Inlet</p> <ul style="list-style-type: none"> <li>• Mode: Splitless</li> <li>• Temperature: 250° C</li> <li>• Injection volume: 1.0 µL</li> <li>• Purge Time: ON at 2.0 minute</li> </ul> <p><b>11.6 Procedure</b></p> <p>11.6.1 Label clean 16 x 125 mm screw cap tubes accordingly, negative, calibrators, control(s) and case sample IDs.</p> <p>11.6.2 Pipet 2 mL of blank blood, calibrators, controls and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes.</p> <p>11.6.3 Add 50 µL internal standard into all tubes and vortex.</p> <p>11.6.4 Add 4 mL deionized water to each tube. Vortex briefly and let stand for 5 minutes.</p> <p>11.6.5 Centrifuge at approx 2000 rpm for 10 minutes.</p> <p>11.6.6 Add 2 mL of pH 6 phosphate buffer to remaining supernatant.</p> <p>11.6.7 Condition the solid phase extraction columns. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.</p> <p>11.6.7.1 Add 3 mL hexane to each column and aspirate on vacuum manifold</p> <p>11.6.7.2 Add 3 mL methanol to each column and aspirate on vacuum manifold.</p> <p>11.6.7.3 Add 3 mL dH<sub>2</sub>O and aspirate.</p> <p>11.6.7.4 Add 1 mL of 0.1 M pH 6.0 phosphate buffer and aspirate</p> <p>11.6.8 Without delay, pour specimens into appropriate SPE columns. Elute from cartridges with ~ 1-2 mL/ minute flow.</p> <p>11.6.9 Wash the solid phase extraction columns:</p> <p>11.6.9.1 Add 3 mL dH<sub>2</sub>O and aspirate at ≤ 3inches of mercury.</p> <p>11.6.9.2 Repeat the dH<sub>2</sub>O wash a second time.</p>	

<b>11 COCAINE, COCAETHYLENE AND BENZOYLECGONINE QUANTITATION AND CONFIRMATION BY SPE AND GCMS</b>	Page 5 of 5
<b>Division of Forensic Science TOXICOLOGY TECHNICAL PROCEDURES MANUAL</b>	Amendment Designator:
	Effective Date: 31-March-2004
<p>11.6.9.3 Wash with 2.0 mL 1.0 M acetic acid and aspirate.</p> <p>11.6.9.4 Add 3 mL methanol and aspirate.</p> <p>11.6.9.5 Add 2 mL hexane and aspirate. Dry the columns at &gt; 10 inches of Hg for at least 10 minutes.</p> <p>11.6.10 Wipe the SPE column tips with Kimwipes®. Place labeled 10 mL conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.</p> <p>11.6.11 Elute drugs by adding 3 mL of freshly prepared dichloromethane/isopropanol/ammonium hydroxide solution to each column. Collect eluate by gravity drain (no vacuum).</p> <p>11.6.12 Evaporate to dryness at approximately 40° C under nitrogen.</p> <p>11.6.13 Derivatize specimens:</p> <p>11.6.13.1 Derivatize by adding 50µL ethyl acetate and 50 µL BSTFA and heat for 15 minutes at 55° C OR</p> <p>11.6.13.2 Derivatize by adding 50 µL of MTBSTFA and heat for 30 minutes at 85° C. Then add 50µL of ethyl acetate.</p> <p>11.6.14 Transfer to GC microvials. Inject 1.0 µL on GC/MS in the SIM mode.</p> <p><b>11.7 Calculations</b></p> <p>11.7.1 Calculate the concentrations by interpolation of a linear plot of the response curve based on peak height (or area) ratios (using the target ions listed under GCMS conditions) versus calibrator concentration.</p> <p>11.7.2 Qualifier ion ratio range. The qualifier ion ratio range is calculated by determining the mean ± 20% (or 2 SD) ion ratio from all calibrators used in the calibrations curve. Each drug has two qualifier ions ratios and each internal standard has one.</p> <p><b>11.8 Quality Control</b></p> <p>11.8.1 See Toxicology Quality Guidelines</p> <p><b>11.9 References</b></p> <p>11.9.1 United Chemical Technologies, Inc. Clean Screen® solid phase extraction procedure for cocaine and benzoylecgonine from whole blood.</p> <p>11.9.2 Spiehler, Vina R. and Reed, Dwight (1985) <u>Journal of Forensic Science</u>, 30:4, 1003-1011.</p> <p>11.9.3 Crouch, Dennis J., et al. (1995) <u>Journal of Analytical Toxicology</u>, 19, 352-358.</p>	